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Role of post-translational modifications, O-GlcNAcylation and Phosphorylation, in neurodegenerative disorders and brain hypometabolism

Role post-translačních modifikací, O-GlcNAcylace a fosforylace, v neurodegenerativnách onemocněních a hypometabolismu CNS

Bachelor´s thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Post-translational modifications are major mechanisms that highly increase the variability in protein function. O-GlcNAcylation and phosphorylation are among the most extensively studied post-translational modifications in research to date. In physiological conditions, O-GlcNAcylation acts as a metabolic sensor that links glucose metabolism to normal neuronal functioning. Reversible phosphorylation is one of the mechanisms that can downregulate metabolism by regulating the rates of flux through metabolic pathways. The impairments in the regulation of these modifications are linked to with neurodegenerative disorders and hypometabolism. This thesis focuses on the crosstalk and correlation between these two modifications, their reciprocal relationship and their mutual impact on their regulations in models of neurodegenerative diseases and disease non-related models.

Keywords: hypometabolism, O-GlcNAcylation, phosphorylation, post-translational modifications, neurodegenerative disorders, hibernation, caloric restriction, memory, learning

Abstrakt

Post-translační modifikace jsou jedny z hlavních mechanismů, které významně zvyšují variabilitu funkce proteinů. O-GlcNAcylace a fosforylace patří mezi nejrozšířenější a nejvíce studované post-translační modifikace. Za fyziologických podmínek O-GlcNAcylace působí jako metabolický senzor, který spojuje metabolismus glukózy s běžnou funkcí neuronů. Reverzibilní fosforylace je jedním z mechanismů, které mohou snížit metabolismus regulováním rychlosti toku metabolickými cestami. Poruchy regulace těchto modifikací jsou spojeny s neurodegenerativními poruchami a hypometabolismem. Práce se zaměřuje na korelaci těchto dvou modifikací, jejich vzájemný vztah a dopad na neurodegenerativní onemocnění a jiné fyziologické modely.

Klíčová slova: hypometabolismus, O-GlcNAcylace, fosforylace, post-translační modifikace, neurodegenrativní onemocnění, hibernace, kalorická restrikce, paměť, učení

List of Abbreviations

Aβ	amyloid β-peptide
AD	Alzheimer's disease
AKT	protein kinase B
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
ATP5A	ATP synthase subunit α
CAG	cytosine-adenosine-guanine
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
Cdk5	cyclin-dependent kinase 5
CJD	Jakob-Creutzfeldt disease
CNS	central nervous system
CoA	acetyl coenzyme A
CR	caloric restriction
CT	computerized tomography
ERK	extracellular signal-regulated kinase
FDG-PET	fluorodeoxyglucose positron emission tomography
GLUT1	glucose transporter -1
GLUT3	glucose transporter -3
GLUTs	glucose transporters
GSK-3β	glycogen synthase kinase 3β
HAT-like	histone acetyltransferase-like
HBP	hexosamine biosynthetic pathway
HD	Huntington's disease
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
MCI	mild cognitive impairment
NFTs	neurofibrillary tangles
O-GlcNAc	O-linked N-acetylglucosamine
OGA	β-N-acetylglucosaminidase
OGT	O-GlcNAc transferase
PD	Parkinson's disease
PHFs	paired helical filaments
PKC	protein kinase C
PKs	protein kinases
PP2A	protein phosphatase 2
PPs	protein phosphatases
PrDs	Prion diseases
PrP	prion protein
PTMs	post-translational modifications
RSCE	repeated short cold exposure

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1 Introduction

Brain hypometabolism, one of the key features of neurodegenerative disorders, is marked by impaired cerebral glucose consumption and utilisation, irregular mitochondrial function and distribution, and oxidative stress. All of these characteristics of defective cerebral metabolism eventually lead to synaptic starvation and neuronal degeneration. Such deficits are key players in cerebral pathophysiology in many neurodegenerative disorders.

At the beginning of the 21st century, the human genome was fully decoded for the first time: it showed an unexpectedly low number of genes when compared to the high complexity of the cells. Post-translational modifications are some of the most significant mechanisms responsible for the high variability of cellular function despite the low number of genes encoding the proteins. PTMs are based on the addition of functional groups to target amino acids, resulting in glycosylation, phosphorylation, acetylation, SUMOylation, ubiquitination, methylation, nitrosylation etc. These can profoundly contribute to increasing protein functionality. This thesis focuses on two of the most widespread and studied PTMs: phosphorylation and O-GlcNAcylation, which have an important role in protein modifications regarding neuronal signalling, synaptic morphology and neurite elongation.

PTMs are highly regulated and have many functions. O-GlcNAcylation acts as a metabolic sensor that links glucose metabolism to normal neuronal functioning. Reversible phosphorylation is one of the mechanisms that can downregulate metabolism through regulating the rates of flux through metabolic pathways. However, abnormalities in their regulation can play a pivotal role in hypometabolism and prompt various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington' disease etc.

The thesis discusses the regulation and correlation between these two modifications, their reciprocal relationship and their mutual impact, with respect to their possible role in future therapy. Research to date has largely focused on investigating the mechanisms and regulation in which these PTMs could have a positive effect on cognitive function. Moreover, different models, such as hibernation, cold therapy or caloric restriction, are being studied where PTMs and different mechanisms as well asi their combination can have a neuroprotective effect.

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2 Brain glucose metabolism

Memory function is built upon highly complex molecular processes. Glucose is the primary cerebral nutrient ensuring proper brain function (Gibbs *et al.*, 1942; Sokoloff and Clarke, 1989; Hyder *et al.*, 2015). It has many pivotal functions, such as ATP production, neurotransmitter and neuromodulators synthesis, and oxidative stress management. Cerebral glucose metabolism and its regulation are the key players in the processes of memory function. The synthesis of acetylcholine, one of the main neurotransmitters, relies on sufficient levels of acetyl coenzyme A (CoA), which is the product of glucose metabolism. Insulin is responsible for regulation of the activity of acetylcholine transferase (reviewed in Dienel, 2019).

Glucose is transported to the brain via the blood-brain barrier and the glucose transporters GLUTs, specifically GLUT-1 located in the endothelium. Once the glucose crosses the barrier thanks to the concentration gradient, it is taken up by GLUT-1 on astrocytes and GLUT-3 and GLUT-4 on neurons (reviewed in Dienel, 2019). Once inside the cells, glucose is metabolised via multiple pathways, one of which is the irreversible phosphorylation. Glucose is phosphorylated to form glucose-6-phosphate , and subsequently enters the glycolytic or tricarboxylic acid pathway. From there, glucose can be glycolysed to form pyruvate, which can be oxidised to CoA. Ultimately, through the tricarboxylic acid cycle, a series of catalytic reactions, ATP is made. The requirements of ATP differ in neuronal cell types according to their functional activity (reviewed in Simpson, Carruthers and Vannucci, 2007).

2.1 Hypometabolism in pathophysiology

Recently, a growing body of evidence suggests that deficient cerebral glucose metabolism manifests itself in the pathophysiology of different disorders. The concept that hypometabolic states and deficiencies in brain metabolic energy play a vital role in Alzheimer's disease (AD) pathology was first suggested by Siegfried Hoyer (Hoyer and Nitsch, 1989; Hoyer, Nitsch and Oesterreich, 1991; Hoyer, 1992, 1998). AD is a chronic neurodegenerative disorder which causes progressive memory deterioration and disruption of other cognitive domains in the human brain (reviewed in Querfurth and Laferla, 2010). Furthermore, a study on structural and functional changes in the brain appearing during Huntington's disease (HD) shows severe glucose hypometabolism in the cortex and in the basal ganglia years before HD shows clinical symptoms (Ciarmiello *et al.*, 2006). Using FDG-PET/CT, a recent study identifies significant patterns of glucose hypometabolism in the striatum in premanifest and manifest HD patients

(López-Mora *et al.*, 2016). Another report suggests a correlation between cerebral hypometabolism and mitochondrial dysfunction in patients with intractable epilepsy independent of the degree of cortical dysplasia (Tenney *et al.*, 2014). Moreover, deficits in glucose metabolism were exhibited in young women with polycystic ovary syndrome. The regional glucose metabolism, which correlated with mild insulin resistance, resembled patterns seen in early AD (Castellano *et al.*, 2015). These findings suggest that mechanisms of cerebral hypometabolism can have various pathophysiological consequences.

2.2 Hypometabolism related neurodegenerative disorders

2.2.1 Alzheimer's disease

Neurodegenerative disorders affect millions of people worldwide and AD is one of the most prevailing ones in the higher age population. AD afflicts about 40 million people globally, accounting for 60-80% of all dementia cases. Most AD cases feature a late-onset and are sporadic in origin. Despite being vastly studied, the actual cause of sporadic AD is still not perfectly understood due to many factors, including genetic, environmental and biological components (Demetrius and Driver, 2013; Hroudová, Singh and Fišar, 2014).

2.2.1.1 The pathology of Alzheimer's disease

The neuropathology of sporadic AD is defined by senile plaques of extracellular fibrillar accumulation of amyloid β-peptide (Aβ) derived from the amyloid precursor protein (Glenner and Wong, 1984), aggregates of the microtubule-associated protein tau in a hyperphosphorylated form, which create a pattern of intracellular neurofibrillary tangles (Grundke-Iqbal, Iqbal and Tung, 1986), and extensive loss of neurons in the brain. Deterioration quickly affects Locus coeruleus, Nucleus basalis of Meynert and layer-II pyramidal neurons of the entorhinal cortex. It then proceeds to the subiculum and hippocampal CA1 subfield. Subsequently, the degeneration affects the isocortical part (Braak and Braak, 1991; Braak *et al.*, 2011; Arendt et al. 1995).

It has been shown that AD has a long preclinical phase in which pathophysiological processes develop years or even decades before any clinical symptoms of dementia appear (Sperling *et al.*, 2011). Recent review shows how different PTMs effect the aggregation of Aβ (Carter and Schaffert, 2020). PTMs responsible for the aggregation are located in the first 28 AA residues (Figure 1). According to some recent studies, accumulation of Aβ and phospho-tau,

immunoreactive for the antibody AT8, is highlighted during very early stages. The plaques occur in the neocortex after the tauopathy in subcortical projection nuclei. These pathological processes, however, are not yet fully understood. Furthermore, it remains uncertain whether these preclinical changes, i.e. the hyperphosphorylated tau in soluble form, ultimately transform into insoluble PHF-tau (reviewed in Arendt, Stieler and Holzer, 2015).

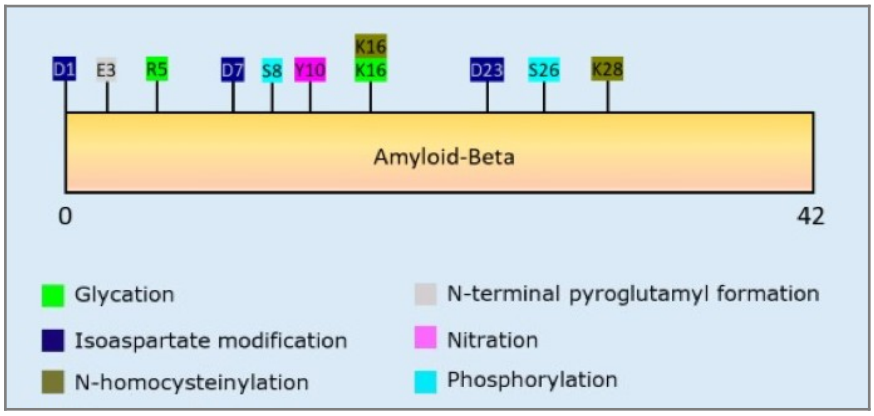

Figure 1: Amyloid β-peptide schematic representation showing PTMs and AA residues. (modified from Carter and Schaffert, 2020)

These pre-symptomatic hypometabolic states occurring in isocortical brain areas can be detected by fluorodeoxyglucose positron emission tomography (FDG-PET) at preclinical stages of AD and can predict the subsequent cognitive decline (Reiman *et al.*, 1996, 2004; Small *et al.*, 2000). As such, greater insight into these preclinical states can lead to the development of therapeutic interventions as well as possible treatment.

2.2.2 Huntington's disease

Huntington's disease, a dominant autosomal neurodegenerative disorder, is caused by unstable expansion of DNA trinucleotide: cytosine-adenosine-guanine (CAG) in Huntington's gene (Kremmer *et al.*, no date). CAG expansion (over 35 repetitions) causes severe brain damage: the progression of the disease is closely linked with major loss of neurons in the striatum. Moreover, the alterations of cortical and subcortical regions and their atrophy are characteristic of HD (Ciarmiello *et al.*, 2006). Interestingly, a recent study has also shown glucose hypometabolism, specifically in the frontal and temporal lobe and the striatum, years before HD shows clinical symptoms (López-Mora *et al.*, 2016), supporting various previous studies (Kuwert *et al.*, 1990; Small *et al.*, 2000; McMurtray *et al.*, 2007; Ciarmiello *et al.*, 2012).

2.2.3 Parkinson's disease

Following AD, Parkinson's disease (PD) is the second most common neurodegenerative disorder in developed countries. Its neuropathology is defined by the loss of dopaminergic neurons in subcortical basal ganglia, specifically the substantia nigra *pars compacta*, and abundant intracellular aggregations of α-synuclein protein. The deposits primarily consisting of α-synuclein are called Lewy bodies. The clinical symptoms of PD include resting tremor, bradykinesia, and loss of postural reflex and rigidity (Poewe *et al.*, 2017).

PD patients with dementia or MCI show signs of severe brain atrophy, especially the loss of grey matter and extensive hypometabolism regions. In patients with PD and MCI, hypometabolism was observed to be more extended (González-Redondo *et al.*, 2014). Cross-validation of three different independent cohorts focused on typical PD-related brain patterns, characteristic for decreased metabolism in the thalamus, cerebellum, pons and motor cortex, and higher levels of metabolism in frontal, temporal, parietal and occipital regions (Meles *et al.*, 2020). The study supports the notion that cerebral hypometabolism is associated with PD.

The formation of Lewy bodies also seems to be greatly influenced by phosphorylation, playing an important role in the fibrillogenesis and neurotoxicity of α-synuclein. Studies have shown that the formations of Lewy bodies in PD mostly consist of α-synuclein phosphorylated at Ser129 (Okochi *et al.*, 2000; Fujiwara *et al.*, 2002; Anderson *et al.*, 2006). In physiological conditions, on the other hand, the majority of the α-synuclein is not phosphorylated *in vivo* (Hirai *et al.*, 2004). There are many PTMs that target α-synuclein, they gather at specific regions of the protein (Figure 2).

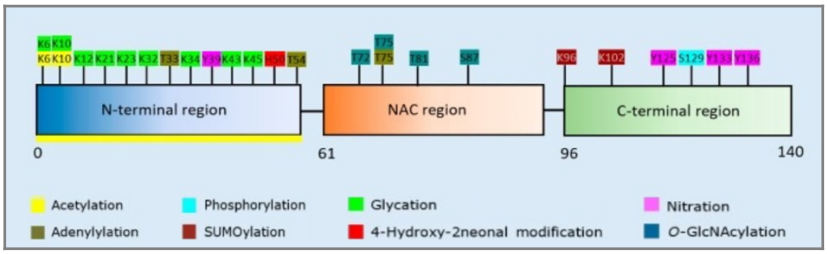

Figure 2:α -synuclein schematic representation showing PTMs and AA residues (modified from Carter and Schaffert, 2020)

2.2.4 Prion disease

Prion diseases (PrDs) are neurodegenerative disorders that cause rapid progressive neuronal decline, specifically neuronal loss, deficits in memory and increased cell death, eventually

leading to a fatal outcome (Moreno *et al.*, 2012). PrDs are triggered by protein misfolding, namely the accumulation of aberrant conformations of infectious scrapie-like proteins called prions (PrP^{Sc}) (reviewed in Aguzzi and Heikenwalder, 2006; Watts and Prusiner, 2014). The most frequent PrD that affects humans is Jakob-Creutzfeld disease (CJD) (Prusiner, 1998).

The cellular prion protein (PrP^{C}) is a membrane-bound glycoprotein with two N-linked oligosaccharide chains of the complex type (Stahl *et al.*, 1987; Turk *et al.*, 1988). PrP^{C} is primarily found in neurons of the brain and spinal cord. Localised predominantly in membrane lipid raft microdomains, it plays an important role in neuronal development and function (reviwed in Westergard, Christensen and Harris, 2007; Bourgognon *et al.*, 2018). More specifically, the protein is attached to the lipid bilayer via GPI anchor and it interacts with various proteins involved in neuronal plasticity, Ca^{2+} signaling and in the regulation of synaptic release at glutamatergic synapses (Chen *et al.*, 2003; Fuhrmann *et al.*, 2006; Krebs *et al.*, 2007; Robinson *et al.*, 2014).

2.2.4.1 The pathology of Prion disease

The cellular PrP is extensively rearranged into a misfolded form of infectious PrP^{Sc} that tends to accumulate. PrP^{Sc} comprises mainly β-pleated sheets which convert the normal cellular PrP into pathological PrP^{Sc} when in contact with the helical structure of PrP^{C} (Kim *et al.*, 2010). The pathogenic and self-propagating PrP^{Sc} then acts as a template for PrP^{C} and it exponentially converts additional PrP^{C} into PrP^{Sc}, eventually leading to neuronal impairment and death (Crespo *et al.*, 2012). Importantly, it has been suggested that PrP^{C} might be involved in the amyloid-β peptide metabolism, a major pathological protein in AD (reviewed in Aguzzi and Heikenwalder, 2006; Westergard, Heather M. Christensen and Harris, 2007).

3 Post-translational modifications in CNS

Post-translational modifications can greatly increase protein functionality. They control the subcellular localisation of proteins, protein conformation and their enzymatic activity, as well as the interaction between different molecular complexes. They thus play an important role in transmitting information throughout the signalling networks in the cell (reviewed in Issad, Masson and Pagesy, 2010).

Due to their pivotal significance in protein function, PTMs are closely regulated. Abnormal post-translational modifications of certain proteins can prompt neurodegeneration, therefore significantly partaking in the pathophysiology of many neurological disorders, including AD, PD, etc. (Didonna and Benetti, 2016). Although these two modifications are among the most studied in research to date, there are few studies thoroughly explaining their significance in neurodegenerative disorders.

3.1 O-GlcNAcylation

One of the most intricate post-translational modifications is glycosylation. It is a highly complex enzymatic pathway with several consecutive steps which occurs in the endoplasmic reticulum or the Golgi compartment. The glycosylation process leads to the construction of multi-functional oligosaccharides bound with proteins. There are three main glycosylations: N-linked glycosylation, O-linked glycosylation and glypiation (Didonna and Benetti, 2016). The following subchapters will mainly focus on O-linked glycosylation.

O-GlcNAcylation is a specific type of glycosylation, which is particular to proteins with O-linked N-acetylglucosamine (O-GlcNAc). The modification is essentially an addition of O-GlcNAc to hydroxyl groups of Ser or Thr residues by intracellular enzyme O-GlcNAc transferase (OGT). β-N-acetylglucosaminidase (OGA) is responsible for removing the O-GlcANc group from the protein (Zachara and Hart, 2006; Hanover, Krause and Love, 2012).

Comprehensive depiction of O-GlcNAc has been rather difficult to achieve due to the protein's low stoichiometry and labile nature. However, a recent proteomics analysis has reported more than 1000 sites of O-GlcNAcylation from human brain tissue samples. Changes in more than 100 O-GlcNAc peptides in AD samples were discovered using multiplexed isobaric labelling with a pooled common reference strategy (Wang *et al.*, 2017).

O-GlcNAc, a derivative of the end product of the hexosamine biosynthetic pathway (HBP), has been proposed to act as a nutritional sensor of brain cells linking glucose and the metabolic status to neurofunction. In recent years, due attention has been given to O-GlcNAcylation as a possible "player" in underlying AD pathophysiology. The attributes of hypometabolism include impaired glucose metabolism, as well as an abnormal function and

distribution of mitochondria. Recent studies suggest that O-GlcNAcylation modifies mitochondrial function, motility and distribution (Pinho *et al.*, 2018, 2019).

3.1.1 Regulation of O-GlcNAcylation

O-GlcNAcylation is regulated by only two enzymes: O-linked β-N-acetylglucos-aminidase (O-GlcNAcase, OGA) and O-linked β-N-acetyl-glucosamine transferase (OGT). OGT catalyses the transport of GlcNAc from ODP-GlcNAc, the donor substrate, to the hydroxyl groups on serine and threonine residues. In contrast, OGA catalyses the hydrolysis of the O-GlcNAc modification (reviewed in Hart, Housley and Slawson, 2007; Yang and Qian, 2017).

OGT is derived from the *OGT* gene and there are three isoforms: nucleoplasmic (ncOGT), mitochondrial (mOGT) and short (sOGT) isoform. The three isoforms have different amino-terminal tetratricopeptide (TPRs) and thus they vary in the length of their c-terminal catalytic domains. Furthermore, the localisation of each OGT isoform inside the cell differs. The ncOGT and sOGT can be found in the nucleus and cytoplasm. Predictably, mOGT is present in mitochondria (reviewed in Hanover, Krause and Love, 2012).

OGA is derived from the meningioma expressed antigen 5 gene and the enzyme has two isoforms, nucleocytoplasmic (ncOGA) and short isoform (sOGA). The ncOGA has two domains, C-terminal histone acetyltransferase-like (HAT-like) domain and N-terminal O-GlcNAc hydrolase domain. The sOGA is located towards the endoplasmic reticulum and lipid droplets and it only carries the hydrolase domain; the HAT-like domain is absent (reviwed in Hanover, Krause and Love, 2012; Ruan *et al.*, 2013).

3.1.1.1 Substrate recognition by OGT

The OGT active site anchors the peptides through side-chain contacts of OGT with the peptide amide backbone (Lazarus *et al.*, 2011). OGT predominantly modifies Ser and Thr residues surrounded by AAs that help to extend the peptide conformation. This proposes a possible effect on OGT substrate recognition through the active site inflicting certain sequence restraints (Pathak *et al.*, 2015).

It has been widely accepted that the substrate selection of OGT is enabled by the N-terminal TPR domain. TPRs could mediate the substrate recognition by creating certain

binding sites that are capable of changing the conformation, and thereby facilitating the access of the substrate to the active site (Yang, Zhang and Kudlow, 2002; Myers, Panning and Burlingame, 2011; Yang and Qian, 2017). Furthermore, TPR can regulate the availability of the active site by rotating a 'hinge' region between the catalytic domain and the TPR, and can thus give access to the active site once the substrate resides at its distinct binding site made from TPRs (Lazarus *et al.*, 2011).

The substrate recognition of OGT could also be facilitated by adaptor proteins, which recruit the substrate to OGT. Activated p38 mitogen-activated protein kinase (MAPK) can enrol OGT to the NFH and subsequently make NFH more soluble by increased O-GlcNAcylation during glucose deprivation (Cheung and Hart, 2008). In this case, MAPK acts as an adaptor protein recruiting the substrate to OGT. Furthermore, during caloric restriction/fasting, Thr-rich HCF1 peptide takes the role of an adaptor protein for OGT. HCF1 directs OGT towards a transcriptional co-activator involved in energy metabolism and increases the level of O-GlcNAcylation, subsequently promoting gluconeogenesis (Ruan *et al.*, 2012). Interestingly, OGA has also been shown to act as an adaptor protein for OGT. It has been suggested that OGA can interact with OGT during high levels of glucose. The HAT-like OGA domain can induce aerobic glycolysis through increased O-GlcNAcylation of PKM2 by OGT (Whisenhunt *et al.*, 2006). This highly specific recruitment of adaptor proteins during certain conditions could help OGT with substrate recognition.

3.1.1.2 Substrate recognition by OGA

The mechanisms of OGA substrate recognition are still not fully understood, due to the lack of crystal structure of eukaryotic OGA. However, there are a few recent studies providing insights into the possible ways in which the mechanisms might work. Li *et al.* suggest that OGA binds in a complex with an inhibitor and in a complex with a glycopeptide substrate. OGA is understood to form an arm-in-arm homodimer where the stalk domain forming one monomer covers the catalytic domain of the other monomer creating a substrate-binding cleft. The residues located on the cleft than interact with the glycopeptide substrate (Li *et al.*, 2017). Moreover, another report describes the structure of OGA as an obligate dimer with intertwined helical-bundle domains leading to residues from both substrate-binding sites. Interestingly, they showed that a flexible loop is a part of the peptide-binding site (Roth *et al.*, 2017). Lastly, in support of previous reports, Elsen *et al*. state that OGA is a flexible dimer forming three distinctive conformations and is characterised by subdomain α-helix swapping. Intersubunit interactions

may affect the binding of substrates with inhibitors. Specifically, α16 plays a major part in substrate binding due to its location close to Tyr645, Tyr641 and Tyr638 catalytic sites. Furthermore, α17 , part of the stalk domain which differs between the two OGA isoforms, is located at the dimer interface through swapping (Elsen *et al.*, 2017). A recent report, however, suggests the presence of substrate promiscuity, similar to protein phosphatases responsible for the removal of phosphorylation, that implements the OGA substrate recognition of myriad O-GlcNAcylated proteins (Yang and Qian, 2017).

3.1.2 Functions of O-GlcNAcylation

Both OGT and OGA can be found in the cytosol and nucleus, although OGT is more usual in the nucleus and OGA is enriched specifically in the cytosol (Kreppel, Blomberg and Hart, 1997; Lubas *et al.*, 1997; Wells *et al.*, 2002). O-GlcNAcylation has many cellular functions, such as signalling dynamics, epigenetic modifications and transcription. It occurs in the Golgi apparatus, ER, and extracellular matrix, as well as in nuclear, mitochondrial, cytoplasmic and plasma membrane compartments (reviewed in Yang and Qian, 2017)

O-GlcNAcylation is involved in regulating transcription by modification of many transcription factors (Jackson and Tjian, 1988). O-GlcNAcylation is necessary for lymphocyte activation (Golks *et al.*, 2007). O-GlcNAc also modifies RNA polymerase II , the C-terminal is both phosphorylated and O-GlcNAcylated reciprocally at Ser2 and Ser5 (Kelly, Dahmus and Hart, 1993). O-GlcNAcylation is also greatly involved in the regulation of epigenetic programmes (Dehennaut, Leprince and Lefebvre, 2014; Lewis and Hanover, 2014; Singh *et al.*, 2015).

O-GlcNAcylation plays a major role in the temporal regulation of cell signalling dynamics, such as the temporal regulation of insulin signalling dynamics. Increased levels of global O-GlcNAcylation in cultured adipocytes hinders insulin-related phosphorylation of AKT, Ser/Thr kinase, which is necessary for cell metabolism (Vosseller *et al.*, 2002). On the other hand, the upregulation of OGA leads to decline in AKT O-GlcNAcylation and enhanced AKT phosphorylation (Soesanto *et al.*, 2008). Interestingly, O-GlcNAcylation modifies Ser473 and Thr308, residues that are also heavily phosphorylated for AKT activation, suggesting a correlation between O-GlcNAcylation and phosphorylation (Shi *et al.*, 2015). O-GlcNAcylation and its signalling is also controlled by temporal regulation. During glucose deprivation, global

O-GlcNAcylation is decreased during the initial hours; later, however, they are considerably increased (Taylor *et al.*, 2008, 2009). In neurons, O-GlcNAcylation is enhanced during the first two minutes of depolarisation, although the PTM later returns to the baseline (Song *et al.*, 2008). Glucose deprivation has been shown to upregulate the expression of OGT and regulate the expression of OGA through a reduction in UDP-GlcNAc levels (Taylor *et al.*, 2008, 2009).

3.1.3 Nutritional regulation

O-GlcNAcylation plays an important role in relation to glucose metabolism as a nutrient sensor. The amount of nutrient available positively correlates with the levels of cellular O-GlcNAcylation via the HBP. Higher levels of ODP-GlcNAc induce *in vitro* O-GlcNAcylation of numerous peptides. Studies have shown the link between higher extracellular glucose levels and higher O-GlcNAcylation. Hyperglycaemia increases levels of cellular O-GlcNAcylation in various tissues *in vivo* (Liu *et al.*, 2000). However, this cannot be described as a simple positive linear correlation. Recent studies have shown that the changing HBP flux is not the only aspect influencing the interlink between nutrient sensitivity and the glycosylation. O-GlcNAcylation on the metabolism coactivator peaked at certain concertation (5mM) and subsequently supressed independently on the following hyper- or hypoglycaemia (Ruan *et al.*, 2012). Such a trend would suggest that the modification levels differ on a substrate-by-substrate basis.

Surprisingly, the levels of cellular O-GlcNAcylation have rapidly risen in nutrient deprivation conditions, which could be explained by upregulation of *OGT* expression. OGT is able to increase O-GlcNAcylation levels even though the UDP-GlcNAc levels are lower (Cheung and Hart, 2008; Taylor *et al.*, 2008, 2009). Moreover, the lack of nutrients can cause cellular stress which leads to the accumulation of unfolded proteins and thus enhances the substrate's affinity to OGT. Therefore, nutrient availability would regulate O-GlcNAcylation by the amount of UDP-GlcNAc present and also by regulating OGA and OGT and their substrates (Yang and Qian, 2017).

3.1.4 O-GlcNAcylation in pathophysiology

The link between altered O-GlcNAcylation and AD has been supported by studies focused on extensive reduction in O-GlcNAcylation levels in AD brain tissue (Liu *et al.*, 2004, 2009). This was first suggested by a Yao and Coleman (1998) study, the results of which reveal a negative interaction between O-GlcNAcylation reduced levels of clathrin assembly protein-3 and the

density of neurofibrillary tangles. A recent study by Yuzwa *et al*. shows that increased O-GlcNAcylation could affect β-amyloid pathology, which is a typical characteristic of AD pathology, in the presence of tau pathology (Yuzwa, Shan, *et al.*, 2014).

The initial stages of AD pathology are indicated by undermined glucose utilisation and availability as a result of the reduced expression of glucose transporter -1(GLUT1) and -3(GLUT3). These impairments coincide with decreased O-GlcNAcylation levels (Liu et al., 2008; Shah et al., 2012). Moreover, O-GlcNAc also modifies protein kinase A catalytic subunits (PKAcs), and PKA-CREB signalling plays a crucial role in the process of learning and memory. Thus the reduction of O-GlcNAcylation of PKAcs in an AD brain might lead to memory and learning deficits (Xie *et al.*, 2016). Furthermore, the loss of OGT in the forebrain leads to neurodegeneration, such as progressive neuronal cell death, activation of neuronal immune response and memory impairments (Wang *et al.*, 2016). Interestingly, O-GlcNAcyation has been shown to have rejuvenating effect on cognitive function in aging brain. Overexpression of neuronal OGT successfully reinstated some of the partly age-related impairments in hippocampus (Wheatley *et al.*, 2020).

3.1.4.1 O-GlcNAcylation during cellular stress

Non-specific O-GlcNAcylation can play a major part during cellular stress. A growing body of evidence promotes O-GlcNAcylation as a key player in the response to changes in protein homeostasis. The stress response pathway is activated once the levels of aggregated unfolded proteins grow. The stress response pathway can block the accumulations of such proteins. The factor of the stress pathway increases cellular O-GlcNAcylation through HBP upregulation, thereby suggesting a major effect of O-GlcNAcylation on the management of unfolded proteins (Wang *et al.*, 2014). There is increasing evidence for the positive effect of O-GlcNAcylation on blocking the aggregations of toxic proteins linked to neurodegenerative disorders, for example α-synuclein in PD (Marotta *et al.*, 2015) or tau protein in AD (Yuzwa *et al.*, 2012).

3.1.4.2 Diminished O-GlcNAcylation and mitochondrial anomalies

Several mitochondrial components undertake O-GlcNAcylation: specifically, the ATP synthase subunit α (ATP5A) has been found to be modified by O-GlcNAcylation at Thr432 in a transgenic mouse model. There is a strong correlation between O-GlcNAcylation levels and hindered mitochondrial function and morphology: a study by Cha and his colleagues shows

modifications of mitochondrial components and ATP5A being a substrate of O-GlcNAcylation. The study gives compelling evidence of ATP depletion and impaired ATPase activity as a result of Aβ blocking interaction between mitochondrial OGT and ATP5A (Cha *et al.*, 2015). The most recent study on this subject also shows O-GlcNAcylation reduction accompanied by the loss of cell viability, thus providing evidence of a strong correlation between O-GlcNAcylation levels, cellular viability and ΔΨm. It demonstrates that SH-S454 exposed to okadaic acid (OA) and streptozotocin (STZ) leads to the collapse of the mitochondrial network (Pinho *et al.*, 2019).

3.1.5 O-GlcNAcylation and phosphorylation of tau protein

Besides phosphorylation, protein tau, the primary component of NFTs, may also be modified by O-GlcNAcylation (Shane Arnold *et al.*, 1996). These two PTMs are both important in the pathophysiology of tau protein. Several consecutive studies showed a reciprocal relationship between phosphorylation and O-GlcNAcylation of tau protein. Furthermore, in an AD brain the hyperphosphorylated tau contained four-fold less O-GlcNAc compared to the non-hyperphosphorylated tau. O-GlcNacylation negatively correlated with phosphorylation of tau at the majority of the phosphorylation sites; specifically the reduced expression of OGT led to increased tau phosphorylation at Thr205 and Thr212 (Liu *et al.*, 2004, 2009; Li *et al.*, 2006; Deng *et al.*, 2008).

Recent evidence supports previous studies and proposes an imbalance between O-GlcNAcylation and phosphorylation in tau. Gatta's research shows a reduction of tau O-GlcNAcylation in the hippocampus in 3xTg-AD mice. The selectively reduced levels of O-GlcNAcylation are associated with tau hyperphosphorylation (Gatta *et al.*, 2016).

However, a recent study contradicts some of the aforementioned well-established findings. Bourré *et al.* report that hyperphosphorylation by ERK2 and normal physiological phosphorylation by the kinase activity of RBE conversely trigger O-GlcNAcylation. They examine tau protein modified by both PTMs and find that phosphorylation by ERK2 precedes O-GlcNAcylation obtained by OGT, meaning phosphorylation actually increases levels already present at O-GlcNAcylation sites. They showed the O-GlcNAcylation pattern on the longest isoform of tau protein and how the regions are targeted by phosphorylation (Figure 3). Moreover, the study shows the improvement of O-GlcNAcylation by increased phosphorylation levels, rather than inhibition (Bourré *et al.*, 2018).

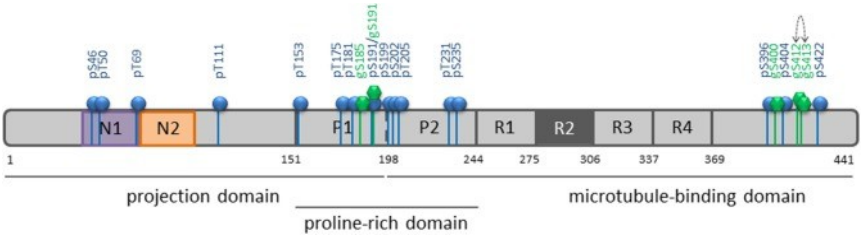

Figure 3: O-GlcNAcylation and phosphorylation pattern of tau protein in its longest isoform using high resolution NMR spectroscopy. Blue bars and dots represent ERK2-mediated phosphorylation sites. Green bars and hexagons represent O-GlNAcylation sites. (Bourré et al.*, 2018)*

A study recent shows increased tau O-GlcNAcylation at Ser400 residue, which suppresses tau aggregation (Yuzwa *et al.*, 2014). OGT inhibitors are revealed to decrease tau O-GlcNAcylation and to facilitate phosphorylation of tau at certain residues (Ser199 and Ser396), therefore favouring tau aggregation (Lim *et al.*, 2015). The hypothesis based on several subsequent studies argues that the reduction of tau O-GlcNAcylation promotes hyperphosphorylation of tau as a result of impaired glucose metabolism (Deng et al., 2009; Liu et al., 2009a; Liu et al., 2004). Furthermore, the inhibition of PP2A caused increased phosphor-tau at certain residues. However, it also showed the difference between the pattern of tau phosphorylation caused by inhibited PP2A and the pattern caused by inhibited HBP. This would suggest, that although both PP2A and O-GlcNAcylation can overlap and regulate tau phosphorylation, they do so with site specification (Liu *et al.*, 2009).

Nevertheless, it is essential to consider that the hypothesis of the imbalance between tau phosphorylation and tau O-GlcNAcylation is based on experiments in which O-GlcNAcylation cycling is alternated by inhibition of OGA activity, which is a catalyst for the removal of O-GlcNAc from proteins. A highly specific OGA inhibitor Thiamet-G has been used to show the suppression of tau aggregation along with JNPL3 transgenic mice. Thiamed-G inhibits OGA, resulting in the reduction of pathological tau in the cerebral tissue (Pinho *et al.*, 2018). Thiamed-G was also used in a following experiment, supporting the claim that Thiamed-G has the capability to induce a substantial build up in overall O-GlcNAcylation levels (Pinho *et al.*, 2019)

3.2 Phosphorylation

Reversible phosphorylation of proteins and enzymes is one of the most abundant PTMs. Phosphorylation modulation of individual proteins in target cells is used by most types of extracellular signals as a way of applying their physiological effects (Li *et al.*, 2013). Protein kinases catalyse the transport of the γ-phosphate group of ATP to the hydroxyl group of target residues. Protein phosphatases balance the catalysation by promoting the hydrolysis of phosphoesther bonds (reviewed in Hanks and Hunter, 1995). Protein phosphorylation appears at Ser, Thr and Tyr residues. Moreover, it provides one of the possible means of regulating and supressing metabolism by limiting the rates of flux in metabolic pathways (Storey, 1987; MacDonald and Storey, 1999; Chen *et al.*, 2001).

3.2.1 Regulation of phosphorylation

Phosphorylation plays a crucial role in myriad cellular functions. Modulation of protein-protein interactions and protein conformation changes are the two main mechanism of phosphorylation control (Nishi, Hashimoto and Panchenko, 2011; reviewed in Nishi, Shaytan and Panchenko, 2014). It is regulated by large classes of protein kinases (PKs) and protein phosphatases (PPs) (Blitzer, Iyengar and Landau, 2005). PKs are responsible for the catalysation of the transfer of γ-phosphate from ATP to specific amino acids, while PPs are primarily responsible for dephosphorylation. Phosphorylation mainly takes place on Ser (85%), Thr (15%) and Tyr (1%) residues (Olsen *et al.*, 2006; reviewed in Humphrey, James and Mann, 2015).

3.2.1.1 Protein kinases

Generally, PKs are classified depending on their sequence, structure and function. There are hundreds of PKs in mammals, including protein kinase A (PKA), protein kinase C (PKC), 5'AMP-activated protein kinase (AMPK), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), etc. These examples of kinases are essential for the regulation of synaptic plasticity, LTP, phosphorylation of NMDARs and AMPARs (Coussens and Teyler, 1996; reviewed in Woolfrey and Dell'Acqua, 2015).

3.2.1.2 Protein phosphatases

The first super-family of phosphatases consists of the largest phosphoprotein phosphatase family including PP1, PP2A, PP2B, PP4, PP5, PP6, PP7, followed by the Mg^{2+} or Mn^{2+} dependent

family (PPM), including PP2C. The second super-family is Tyr phosphatases. The third is the aspartate-based PP family (reviewed in Moorhead, Trinkle-Mulcahy and Ulke-Lemée, 2007; Y. Tweedie-Cullen, M. Brunner and M. Mansuy, 2012). NMDAR-dependent LTD relies largely on phosphatase activity, such as calcineurin (CaN) (Mulkey *et al.*, 1994), protein phosphatase 1 (PP1) (Mulkey, Herron and Malenka, 1993), or protein phosphatase 2 (PP2A) (Mauna *et al.*, 2011).

3.2.2 Phosphorylation and cellular signalling

Following the influx of calcium ions into the neurons, PKs and PPs are activated and the cascade of biochemical reactions is induced, which eventually leads to neuronal signalling.

The different affinity towards Ca^{2+} regulates the activity of both calcium dependent PKs and PPs (Lee, 2006). Together with the calcium affinity, numerous inhibitors and targeting partners, such as adaptor proteins, scaffolding, and anchoring control the activation of the enzymes. These protein partners enable substrate recognition by recruiting the enzymes to protein complexes and helping them to get closer to the substrate, essentially making them more selective towards the accessibility of the substrate (Faux and Scott, 1996). Thus, phosphorylation is affected by the balance of PKs and PPs and also by cellular restricted localisation, which is necessary for the coordination of signalling cascades including short-term and long-term signalling processes.

3.2.3 Multisite protein phosphorylation

The regulation of protein function by phosphorylation is not limited to a simple switch mechanism, in which PK phosphorylates its substrate and changes the activity. Multisite protein phosphorylation is a common mechanism that regulates protein function and cell signalling and actually increases the possibilities for regulation of protein function. A single protein can often be phosphorylated on multiple sites (5 different sites on average) by multiple PKs, which is evidenced by MS-based proteomics (Huttlin *et al.*, 2010; Olsen *et al.*, 2010; Sharma *et al.*, 2014). Multisite protein phosphorylation is the predominant mechanism which regulates cellular signalling. It predominantly regulates the unstructured domains (Tyanova *et al.*, 2013) and occurs mainly at protein-complex interfaces, causing significant changes in protein structure (Nishi, Hashimoto and Panchenko, 2011).

Interestingly, phosphorylation of a single site might represent a ‘priming’ step before the kinases phosphorylate other sites, again presenting a possible mechanism for enabling signal integration; glycogen synthase kinase 3β (GSK3β) presents one such example. When the +4

position of the substrate is phosphorylated, further hyperphosphorylation of the substrate follows (Dajani *et al.*, 2001). Multiple different kinases can target one substrate to act as metabolic signal integrators.

3.2.3.1 Signal integration

Proteins can be phosphorylated on multiples sites by different kinases. Possible overlapping of different kinases can lead to various effects on function. These integrators are vital for metabolic signalling, such as the 6-phospho-fructo-2-kinase/fructose 2,6-biphosphate (PFK2) enzymatic family, heat shock factor 1, Rab GTPase activating protein AS160 etc, which regulates insulin-dependent trafficking of GLUT4. PKC and 5'AMP-activated protein kinase (AMPK) can also control AS160 and through GLUT4 regulate glucose uptake depending on the insulin levels (Thong, Bilan and Klip, 2007; Kjøbsted *et al.*, 2015). PFK2 family of enzymes can control the alternation between glycolysis and gluconeogenesis, acting as a switch (Murray *et al.*, 1984).

3.2.4 Metabolic regulation

Similarly to other PTMs, such as previously mentioned O-GlcNAcylation, phosphorylation and metabolism are closely interlinked (Du *et al.*, 2008). In reference to hypometabolism, the most-studied role of phosphorylation is the neuronal cytoskeleton and cellular localisation, the assembly and abnormal aggregation of neurofilaments (reviewed in Muñoz-Lasso *et al.*, 2020). Nevertheless, it is important to note the nutrient-dependent phosphorylation, which directly affects the glucose uptake and ATP consumption (Hardie, 2011; Hardie, Ross and Hawley, 2012).

AMP-activated protein kinase (AMPK) is one of the more extensively studied examples of nutrient-dependent phosphorylation. It maintains the cellular energy homeostasis and has three subunits: catalytic subunit α and regulatory subunits β and γ (reviewed in Assefa *et al.*, 2020). When energy expenses are high, AMPK can phosphorylate its targets, resulting in increased ATP generation and inhibiting pathways responsible for ATP consumption (reviewed in Hardie, Ross and Hawley, 2012). AMPK largely regulates glucose uptake through GLUT4 and GLUT1.Through the hypothalamus, it can also regulate the metabolism and energy-balance in the whole body (reviewed in Hardie, Ross and Hawley, 2012). It is mainly activated by AMP/ ATP ratio and reactive oxygen species.

Diacylglycerol, capable of activating PKC, and cAMP, which is able to activate PKA, is another example of a metabolic product that is able to induce the activity of different PKs. Glycolysis is another metabolite which can act as a signalling modulator (Trefely *et al.*, 2015).

3.2.5 Phosphorylation in pathophysiology

3.2.5.1 Tau hyper-phosphorylation

Tau protein occurs in six isoforms and is localised mainly in neuronal axons. Its main function is regulating neuronal transport and stabilising microtubules. Hyperphosphorylation prevents tau protein from attaching to the microtubule network and, instead, induces the formation of aggregated paired helical filaments (PHFs), the building blocks of intracellular neurofibrillary tangles (NFTs) (Medina, Hernández and Avila, 2016; Jouanne, Rault and Voisin-Chiret, 2017). This is one of the main hallmarks of AD pathophysiology; nonetheless, it is worth noting that the formation of NFTs and the subsequent progression to AD is only specific to particular isoforms of tau and sites of phosphorylation. As reviewed in a recent report (Liu *et al.*, 2016; Carter and Schaffert, 2020), evidence which suggests that the phosphorylation of specific tau isoforms might decrease aggregation and that there are various PTMs that effect the aggregation, shown in Figure 4.

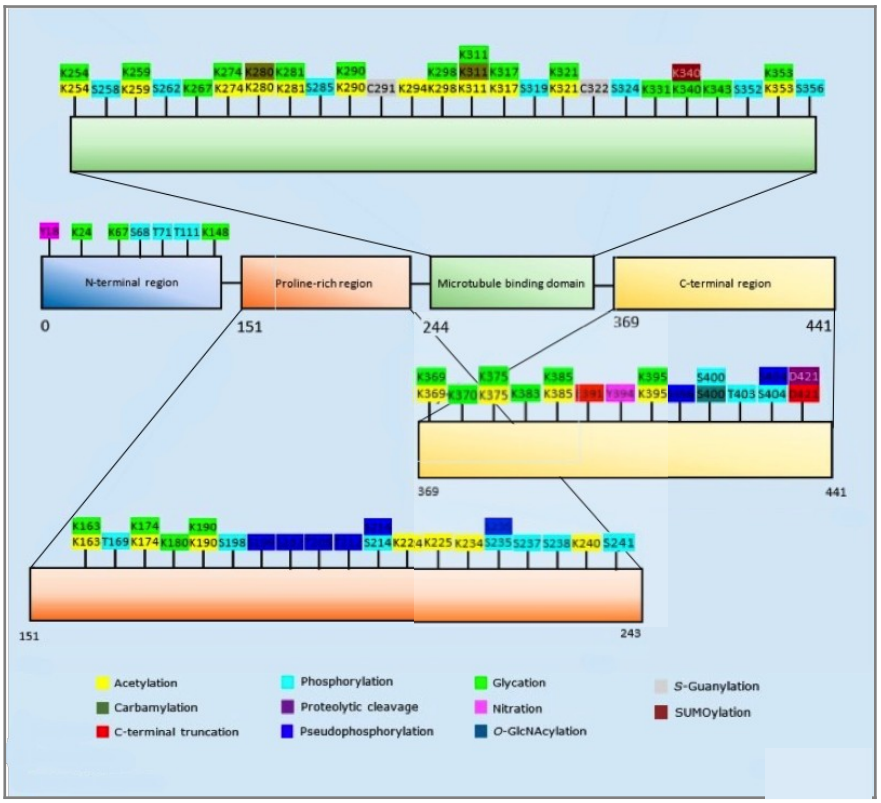

Figure 4: Tau protein schematic representation showing PTMs and AA residues. (modified from Carter and Schaffert, 2020)

Levels of hyperphosphorylation are regulated by the balance between phosphorylation and dephosphorylation. Tau phosphorylation is regulated by proline-directed tau kinases, including ERK, GSK3β and Cdk5, and non-proline-directed tau kinases, including AMPK, CaMKII and MARK1. Different kinases regulate the phosphorylation of tau at different sites. At Ser396, phosphorylation reduces the function of microtubules (Wagner *et al.*, 1996). It has been suggested that GSK3β is the major kinase responsible for phosphorylation at its site (Duka *et al.*, 2013). Dephosphorylation of tau is regulated by phosphatases, mainly PP2A

3.2.5.2 α-synuclein phosphorylation

Alpha-synuclein is a cytosolic protein found at presynaptic terminals of neurons in the CNS. The protein's function is not yet fully understood, however it has been suggested that it plays a significant role in synaptic signalling and possible neurotransmitter release (Bendor, Logan and Edwards, 2013). There are various kinases that can phosphorylate the five α-synuclein's phosphorylation sites (Ser87, Ser129, Tyr125, Tyr133, Tyr 136). These kinases include casein kinases 1 and 2 (CK1 and CK2), polo-like kinases and G-protein coupled receptor kinases (Waxman and Giasson, 2011). Only about 4% of α-synuclein is phosphorylated on the Ser129 level in physiological conditions, whereas about 95% of phosphorylated α-synuclein appears in Lewy bodies, which are accumulations of α-synuclein protein and a major hallmark of PD (Anderson *et al.*, 2006).

3.2.5.3 AMPK in hypometabolism

Studies show that the mechanisms of autophagy are impaired in AD (reviewed in Li, Zhang and Le, 2010; Wei *et al.*, 2019). AMPK is a major positive regulator of autophagy and several studies suggest that AMPK activators play a role in autophagic clearance of Aβ (Zhao *et al.*, 2015; Park *et al.*, 2016). Moreover, recent research suggests that various AMPK activators are able to decrease tau hyperphosphorylation by inhibiting GSK-3β (Park *et al.*, 2012; Kim *et al.*, 2017; Kornelius *et al.*, 2017). AMPK activation can also induce activation of PP2A and thus reduce tau phosphorylation (Kim *et al.*, 2015). A number of studies highlight the involvement of AMPK in PP2A regulation: more specifically, AMPK phosphorylation on Ser298 and Ser336 residues have been found to increase PP2A activity (Kim *et al.*, 2009, 2015; Chen, Li and Zhu, 2016).

A recent study suggests an isoform-specific AMPAKα dysregulation in AD pathology. The upregulation of AMPAKα1 induced AD cognitive and synaptic impairments. The report indicates that the expression of AMKPα1 isoform was increased in the hippocampal neurons of both familial and sporadic AD patients. Furthermore, when the expression of AMKα1 was decreased in the hippocampus and cortex, the synaptic deficits and memory decline were improved (Zimmermann *et al.*, 2020).

Several studies have shown that activation of AMPK can induce AD. AMPK can impact the pathogenesis of AD in various ways (Figure 5). There are several reports suggesting that AMPK activation reduces the oxidative stress and tangle and plaque formation. However, a substantial number of studies highlight the negative effect of AMPK activators. Thus, the relationship of AD and AMPK is an important area for future research (reviewed in Assefa *et al.*, 2020).

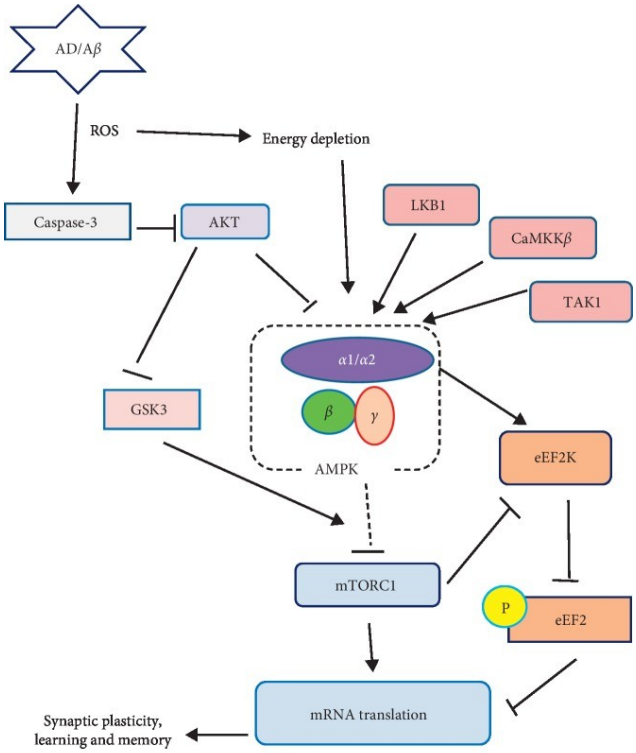

Figure 5: Summarizing schematic model of the regulators and effectors of AMPK and link to AD. Arrows denote activation and blunted lines indicate inhibition. (Assefa et al.*, 2020)*

4 Neuroplasticity during down-regulation of metabolism

4.1 Downregulation in hibernation

Hibernation, a behavioural adaptation adopted by many mammalian species in extreme environmental conditions to save energy expenses, is characterised by a physiological decrease

in metabolic rate accompanied by a decrease in body temperature, including brain temperature. The majority of the energy supply of the mammalian brain is used for the maintenance of neuron resting potential and keeping the integrity of cells (Du *et al.*, 2008). During torpor, glucose metabolism is significantly decreased to 1-2% of an active animal's metabolic rate (Frerichs *et al.*, 1995), and thus the neuronal activity is low in deep torpor and may hinder the preservation of neuronal connections (Kavanau, 1997). Even when the cerebral temperature drops to 2-3°C above the ambient temperature, organisms are still capable of arousal without any major impairments. The structure of neurons during torpor is generally altered: the dendrite spines are shortened and less branched (Popov *et al.*, 1992; Magariños *et al.*, 2006). In the hippocampus, CA1 and CA3 pyramidal cells show spine loss and retraction during torpor. Neuron firings are reduced due to the decreasing temperature and incoming hibernation (Kilduff, Sharp and Heller, 1982). Although neuronal firings are decreased, the basic function and homeostasis of the subcortical brain is sustained (reviewed in Horowitz and Horwitz, 2019). Most importantly, within hours, the dendrite's morphology and structure is completely recovered (Popov *et al.*, 1992).

4.1.1 Reversible phosphorylation during hibernation

Reversible phosphorylation is one of the major mechanisms that facilitates the downregulation of metabolism and thermogenesis during hibernation. Reversible phosphorylation can regulate the rates of flux through metabolic pathways (Chen et al. 2001; MacDonald and Storey 1999; Storey 1987). It also has a major effect on tau protein (Arendt et al., 2003) and synaptic membrane proteins that are associated with synaptic plasticity (Walaas and Greengard, 1991). During torpor, Ser and Thr residues of tau are phosphorylated, directly affecting synaptic plasticity. Extensive phosphorylation is common in neurodegenerative disorders, albeit it is highly tolerated during hibernation and it promptly reverses upon arousal (reviewed in Arendt, Stieler and Holzer, 2015). Interestingly, the formation of hyperphosphorylated tau in CA3 in the hippocampus corresponds with the decrease in synaptic connections in mossy fibre terminals. During hibernation such correlation would suggest a connection between tau phosphorylation and During hibernation, tau is highly phosphorylated, forming PHF-like epitopes, which are, however, completely tolerated during the state of torpor, completely reversible upon arousal and not associated with fibril formation. Tau phosphorylation is regulated by different phosphatases and kinases. The kinases include cyclin-dependent kinase 5 (cdk5), MAPK, stress-activated kinases (SAPJ/JNK) and glycogen synthase kinase-3β (GSK-3β). Phosphatases comprise

mainly protein phosphatase 2A (Arendt *et al.*, 1998; reviewed in Sergeant *et al.*, 2008). Moreover, the phosphorylation of tau can be accompanied by decreased connections between synapses in mossy fibre terminals, further linking synaptic plasticity with tau phosphorylation during hibernation (Arendt and Bullmann, 2013).

Furthermore, phosphorylated tau regulates the postsynaptic targeting of Src-kinases Fyn. It is possible that during hibernation hyperphosphorylated tau does not bind to microtubules, but instead translocates to spines, and through NMDAR impairs synaptic plasticity (Lee *et al.*, 1998; Klein *et al.*, 2002; Lee, 2005). During hibernation, a gradual decrease in body temperature takes place, which results in increased depolarisation and excitability of hippocampal neurons, as well as others. Overall increased neural activity when entering hypothermia is suggested to be NMDA-dependent. Due to the hyperphosphorylated tau, the decrease in NMDAR's function might help the brain to enter torpor. Considering its effect on NMDAR, tau phosphorylation could efficiently regulate the neuronal activity under controlled conditions. However, if prolonged hypometabolic conditions persist, such modifications turn pathological (reviewed in Arendt, Stieler and Holzer, 2015).

It has been shown that PPs decrease exponentially, whereas PKs decrease linearly in low temperatures. PP2A has demonstrated a greater ability to dephosphorylate tau compared to PP1 and PP2B (Planel *et al.*, 2004). The effects of reduced PP2A activity are able to induce hyperphosphorylation of tau. PPA2 might be a major tau phosphatase *in vivo*, while in AD, PPA2 activity is decreased.

A recent review suggests a model of CA1 pyramidal neurons in the hippocampus of small hibernating mammals, where in torpor extreme neuroplasticity occurs and modifies CA1 neurons physiologically and anatomically. Tau phosphorylation during torpor is associated with reduction and retraction in dendritic spines and therefore marks anatomical plasticity that creates a smaller and more compact form with lower energy expenditure (Horowitz and Horwitz, 2019).

4.2 Cold adaptations

Impaired thermoregulation is considered as a characteristic of metabolic disorders. According to a recent study, repeated short cold exposures (RSCE), as a way of stimulating brown adipose tissue diminish hypothermia-induced tau hyperphosphorylation and improve metabolic

shortages in 3xTg-AD mouse models (Tournissac *et al.*, 2019). The increased brown adipose tissue thermogenesis through RSCE improved glucose metabolism (Hanssen *et al.*, 2015; Wang *et al.*, 2015).

Furthermore, despite the body temperature significantly decreasing, models introduced to RSCE for 4 weeks did not show increased levels of insoluble phospho-tau. In fact, after being exposed to cold temperatures (4 °C, 24 h), levels of phosphor-GSK3β increased. Although they stayed equivalent to the control level upon RSCE, the levels of phosphor-JNK and phospho-AKT also increased after the cold exposure. There were no major changes in the levels of PP2A, MAPK, CAMKII. This fact indicates that controlled exposures to cold temperatures do not induce pathological tau accumulations; on the contrary, they present a possible therapeutic strategy against metabolic disorders, including dementia and neurodegenerative disorders (Tournissac *et al.*, 2019).

4.3 Caloric restriction

Caloric restriction has a benefitial effect on cognitive functions and supports the survival of neurons (reviewed in Fusco and Pani, 2013). Intermittent fasting, a type of calorie restrictive diet, has been shown to selectively increase the expression of the NR2B subunit in NMDAR in adult mice (Fontán-Lozano *et al.*, 2007). Moreover, the majority of proteins affected by CR are involved in mitochondrial activity (Poon *et al.*, 2006). However, recent research does not show a direct correlation between dietary constriction and the reduced function of glutamatergic receptors (Kumar, Yegla and Foster, 2018; Rojic-Becker *et al.*, 2019). These results stand in contrast with previous studies, where the subunit Glu2A of NMDAR was found to be reduced by caloric restriction (Eckles-Smith *et al.*, 2000; Monti, Virgili and Contestabile, 2004; Shi *et al.*, 2007; Adams *et al.*, 2008).

A recent study investigated how CR could affect memory, Ca^{2+} homeostasis and hippocampal O-GlcNAcylation, a major PTM that has been linked to AD and diabetes (Jeon *et al.*, 2016) in the ob/ob mice model of obesity-induced diabetes. The findings show that CR improved learning processes in ob/ob mice and notably increased hippocampal levels of O-GlcNAc. At the same time, OGT decreased the expression CAMKII and phosphorylated tau (Jeon *et al.*, 2016). Furthermore, in accordance with other studies, CR abated the metabolic

impairments, improved insulin sensitivity and caused weight loss (Sloan *et al.*, 2011; Tsutsumi *et al.*, 2011; Jeon *et al.*, 2016).

Additionally, present work shows a possible correlation between 2-deoxyglucose (2DG) mimetic of caloric restriction and improved brain function. 2DG decreases the uptake of cellular glucose due to its competition with glucose for the same transporter when entering the cell, and it modulates Cdk5 and GSK3β, kinases responsible for tau phosphorylation (Cruz *et al.*, 2003; Noble *et al.*, 2003; Lei *et al.*, 2011; Bele, Gajare and Deshmukh, 2015). Therefore, 2DG can be used to mimic CR. The results showed low immunofluorescence signals for Cdk5, GSK3β, phospho-tau at Ser235 and Ser262, thereby implying decreased levels of tau phosphorylation (Bele, Gajare and Deshmukh, 2015)..

5 Conclusion

The majority of proteins are post-translationally modified, which helps them to greatly increase their protein function. In this thesis, the focus has been on the two most widespread and studied post-translational modifications, phosphorylation and O-GlcNAcylation. The thesis has highlighted their connection to impaired cerebral glucose metabolism, their involvement in the pathophysiology of various neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Prion disease, Huntington's disease, and, significantly, their mutual crosstalk and correlation.

Despite its great involvement in neurodegenerative diseases and cognitive impairment, mounting evidence shows that tau hyperphosphorylation need not be detrimental in all cases. On the contrary, high reversible phosphorylation present during the torpor stages of hibernation shows positive effect on the regeneration of the dendrite's structure. Furthermore, current research focuses on linking the cold shock proteins and their neuroprotective properties with tau phosphorylation in postsynaptic density. Reversible phosphorylation is associated with cold-shock proteins that are responsible for the synaptic regression upon torpor, followed by reinnervation of neurons after arousal. Phosphorylation of tau present at postsynaptic density is suggested to be linked with the cold-shock related phosphorylation, pointing to a new direction of research to find potential therapy targets.

O-GlcNAcylation seems to have a predominantly beneficial effect on neuroplasticity. There is increasing evidence for the positive effect of O-GlcNAcylation on blocking the folding and aggregations of toxic proteins linked to neurodegenerative disorders, such as tau protein in Alzheimer's disease and α-synuclein in Parkinson's disease. Furthermore, increased levels of hippocampal O-GlcNAcylation using caloric restriction improve the learning process and cognitive function. Similarly, the enhanced O-GlcNAcylation in the hippocampus had a rejuvenating effect on cognitive function and partly mended learning and memory impairments in the aging brain.

The various studies discussed in this thesis suggest a reciprocal relationship between phosphorylation and O-GlcNAcylation of tau protein. A reduced expression of OGT leads to increased tau phosphorylation at certain sites. Increased tau O-GlcNAcylation at Ser400 residue

suppresses tau aggregation. OGT inhibitors decrease tau O-GlcNAcylation, facilitating phosphorylation and favouring tau aggregation. However, recent research shows that phosphorylation can increase levels of O-GlcNAcylation at certain sites, and increased phosphorylation improves O-GlcNAcylation. The exact mechanisms that cause decreased O-GlcNAcylation, which leads to the onset of AD, are not yet fully explained. As such, investigation into the precise mechanisms and ways of reciprocal regulation of these two post-translational modifications, is an avenue for further research.

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